Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5005–5009, July 1989
Genetics

Transcriptional bias: A non-Lamarckian mechanism for substrate-induced mutations

(mutation rates/stationary-phase bacteria/evolution/carcinogenesis)

BERNARD D. DAVIS

Bacterial Physiology Unit, Harvard Medical School, Boston, MA 02115

Contributed by Bernard D. Davis, January 30, 1989

ABSTRACT **In bacterial cultures in the stationary phase, substrates can selectively stimulate mutations that lead to their own utilization, but because of apparent conflict with the neo-Darwinian view of evolution the phenomenon has encountered widespread resistance. Building on further evidence for this process, Cairns *et al.* [Cairns, J., Overbaugh, J. & Miller, S. (1988) *Nature (London)* 335, 142–145] have suggested a Lamarckian mechanism of directed mutation. This paper proposes an alternative mechanism: transcription induced by the substrate introduces a bias in the random process of mutation, because the resulting single-stranded regions of DNA are more mutable. This stimulation of adaptive mutations by the environment has implications for evolution similar to those of directed mutation, but without contradicting the central "dogma" of molecular genetics. In addition, in eukaryotic cells a mutagenic effect of induction on protooncogenes could contribute to the stimulatory effect of proliferation on carcinogenesis.**

Cairns *et al.* (1) have recently presented several kinds of evidence that a potential substrate can cause stationary-phase bacterial cultures to accumulate, selectively, mutations that allow its use. They further concluded that these findings are inconsistent with the neo-Darwinian view, in which adaptive mutants emerge only by random mutation followed by selection at the level of the phenotype. Instead, they suggested that a variant messenger molecule, produced by a directed process or perhaps at random, might undergo reverse transcription when its product proves useful.

This challenging article has provoked extensive discussion. In an accompanying comment, Stahl (2) suggested that replication, dependent on energy provided by the substrate, might stabilize an otherwise transient mutation in the DNA; and many readers offered additional suggestions, listed in a reply by Cairns (3). Most of these rejected a specific influence of substrate on mutation rate, suggesting instead that differences in survival or in growth rate of mutant and parent could reconcile the findings with a neo-Darwinian interpretation.

None of these suggestions seems to me to counter convincingly the evidence for a selective influence of substrates on mutation rates. At the same time, if we accept this finding it does not follow that we must also accept the challenging conclusions to which it led Cairns *et al.* (1): that "cells may have mechanisms for choosing which mutations will occur"; that we may be dealing here with the inheritance of acquired characteristics; and that this process may even depend on information transfer from protein to nucleic acid. These propositions are difficult to reconcile with known properties of the genetic material. I shall here describe an alternative mechanism that eliminates that dilemma: by stimulating selective transcription the environment can impose a bias on the "random" process of mutation, thus increasing the frequency of adaptive mutations.

Although such a bias would be mechanistically quite different from choosing mutations (i.e., specifically directing changes in sequence), the implications for evolution could be much the same. Hence, even if Cairns's tilt toward Lamarckism should have to be rejected it has served a very useful purpose, stimulating us to reconsider a long series of stubborn facts that have suffered neglect because they seemed hard to reconcile with neo-Darwinian doctrine.

Accelerated Mutations in the Stationary Phase

In perhaps the earliest hint of the problem, Ryan (4) observed that *Escherichia coli* in the stationary phase yielded adaptive mutations at rates beyond what might be expected from their frequency per cell division in growing cultures. Later, Hall (5) investigated the problem within a framework of molecular biology: a strain with a deletion in the gene for β-galactosidase (*lacZ*) cannot restore the enzyme, but when it is incubated for many days in the presence of lactose it evolves another enzyme for utilization of that substrate. Moreover, the appearance of such mutants was found, surprisingly, to require two mutations: one in the gene for the new enzyme (*ebg*), which had evidently evolved with some other specificity, and the other in its repressor (whose wild-type form makes the enzyme "cryptic"—i.e., prevents its formation until the repressor mutation). Yet neither of the single mutations provides any detectable advantage, which would have facilitated successive selection; and the product of their observed rates, in the absence of lactose, would be far too low to account for the double mutations (5).

In a similar pattern, development of the unusual ability to utilize citrate as a carbon source also required two mutations in *E. coli*, and they did not appear until after 14 days of incubation (6). Both these findings seem to violate an ancient principle governing multiple mutations, which was introduced by Paul Ehrlich: combined therapy with two drugs with different modes of action will prevent the emergence of resistant mutants, since their frequency should be the product of the frequency of the mutations to resistance to each drug.

Studies of Shapiro (7), supplemented by Cairns *et al.* (1), further extended this investigation to mutations that excised a transposable segment: bacteriophage Mu inserted into a fusion between the *ara* promoter and the *lac* operon. Incubation for several days with arabinose and lactose (but not with either alone) led to frequent excision of the Mu and hence to colonies that could grow on that medium. The kinetics were remarkable: colonies did not begin to appear until after 4 days, and over the next 2 weeks their rate of further appearance rapidly increased. Up to 39% of the plated

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: IPTG, isopropyl β-D-thiogalactoside.

cells eventually produced fusion clones—yet cultures grown without starvation produced none.

Recently, Hall (8) has provided an especially thorough analysis, in cells that would require a double mutation before they could utilize β-glucosides. One mutation excised an insertion sequence that had been placed in a gene for the transport and phosphorylation of β-glucosides, while the other, a point mutation, eliminated repression by the regulator of that gene (much as with the cryptic *ebg* gene described above). After 2–3 weeks of incubation on plates in the presence of the β-glucoside salicin, $\approx 10^{-8}$ of the cells developed both mutations and then grew at the expense of that substrate.

Quantitative analysis for the two individual mutations further showed that colonies 8–12 days old contained a remarkable frequency (1–10%) of cells with only the excision. Yet reconstruction experiments showed no growth advantage of cells with this mutation. Accordingly, their high frequency in the aged colonies represented a burst of independent excisions, providing a large population within which the less frequent second mutation, in the regulator gene, then provided an occasional adaptive clone.

In another, briefly noted example, the presence of maltodextrins too large for ready entry into the cell promoted, after prolonged incubation, a mutation (in an outer membrane porin) that allowed such entry (9).

Cell Turnover in the Stationary Phase?

One conclusion from these studies is no longer controversial: bacteria not only accumulate mutants in growing cultures, with classical kinetics, expressed in terms of generations; they also accumulate them, without net growth, in stationary cultures. This is not surprising. For while the important role of spontaneous, random mutations in bacteria, at a fixed rate per generation, was discovered before the molecular mechanisms of mutation were known, we now know that some mutagens are chemically reactive with nonreplicating DNA (e.g., alkylating agents, radiation), while others act only on replicating DNA (e.g., base analogues). Spontaneous mutations should therefore also be expected to occur in nonreplicating as well as in replicating DNA. The former class cannot be elegantly quantitated, and they have been much less attractive to investigators. Nevertheless, they may be much more important in evolution, because bacteria in nature are so often nutritionally deprived.

More interesting are the problems of the delay and then the apparently high rate of mutation in the stationary phase, the much greater rate of excision than of point mutation (7, 8), and the stimulatory effect of substrates. As a background for discussing these genetic problems we should review briefly some features of the physiology of stationary cells.

To explain mutations in stationary-phase cells—and even more to explain the formation of new, adaptive enzymes by these cells—it has often been assumed that in such cultures some cells multiply at the expense of nutrients supplied by the lysis of other cells ("cryptic" growth). In fact, however, this assumption has very little support. While lysis no doubt occurs eventually in all aging cultures, and early with some fragile organisms, it is now clear that it is not necessary, or prominent, in the activities just described in the hardy enterobacteria. Ryan's early studies on rates of mutation of His^- to His^+ provided evidence that very little DNA synthesis occurs in the stationary phase (10): for example, mutations accumulate over long periods of time in spheroplasts, which cannot divide (11). (These experiments, of course, do not exclude replication or repair of short segments of DNA.) More decisive is the direct evidence against significant lysis: in a starved *Aerobacter aerogenes* culture essentially all the cells retained an intact osmotic barrier even after the viability count had dropped by 80% (12).

Later studies shed further light on the activities of cells in the presumably dormant stationary phase. Cells entering that phase complete their current round of DNA replication (thus increasing the stability of the DNA), and they then segregate their chromosomes by further cell division, yielding cells with only one, complete chromosome and with one-fourth the mass of the average cell in rapid growth (13). Moreover, during the stationary phase the composition of the cell changes extensively, in ways that are of great value for bacteria in their adaptation to a life in nature of alternating feast and famine.

For example, resumption of growth after starvation often requires the induction of enzymes to attack new substrates and to regenerate repressed biosynthetic pathways. Apart from these variable specific adaptations, starvation of *E. coli* cells induces the formation of ≈30 proteins, some novel, in several successive temporal waves; and mutations show that some of these promote survival during starvation (14). Moreover, many antibiotics and other secondary metabolites, produced only during the stationary phase, require induction of new enzymes (15). Finally, starvation induces formation of a secreted bacteriocin (microcin B17), but the regulatory mechanism leading to its increased transcription has proved elusive (16). These changes are evidently fueled not by cryptic growth at the expense of lysis, but by an elaborately regulated intracellular digestion of constituents that can be spared; among these, the ribosomes are particularly prominent (ref. 17 and references therein).

The Role of Substrate in Stationary-Phase Mutations

Let us return to the main problem: the apparently specific induction of adaptive mutations in nongrowing cultures by potential substrates, and especially the appearance of double mutants. Hall (8) has suggested a general explanation: "Physiological regulatory feedback loops could modulate the probabilities of mutations at specific loci when the cell is under stress."

I wish to suggest a more specific mechanism within this framework. Transcription requires separation of the strands and therefore some degree of increased exposure of short regions of single-stranded DNA; these strands are more vulnerable than double-stranded DNA to damage by enzymatic, chemical, or thermal attack; and damage leads to erroneous replication or to error-prone repair mechanisms. Accordingly, the transcription induced by substrates should create regions of increased mutability.

Several kinds of evidence support this conclusion. In transcription the binding of RNA polymerase to the promoter *in vitro* not only blocks access of certain amino groups to methylation by dimethyl sulfate but also makes others more reactive. In addition to this effect on a double-stranded region, the "bubble" that the enzyme unwinds, of about 12 bases, exposes *N*1 of adenine, which is ordinarily paired; and methylation of this group shows that it is not protected by the enzyme (18). Though the disposition of the two single DNA strands in space is unknown, the untranscribed strand may well be more reactive than the one that is paired with nascent RNA.

Many observations have demonstrated the greater vulnerability of single-stranded DNA. For example, *in vitro* it exhibits a >100-fold greater rate of depurination (19) or of deamination (20) than double-stranded DNA. Similarly, a review of chemical mutagenesis (21) simply stated that "Most, if not all, mutagens are much more reactive in single-stranded nucleic acids, so that these regions are probably preferentially modified in replicating DNA."

Studies in cells have provided more direct evidence. Induction of the *lac* operon increased 2-fold the reversion of a Lac$^-$ frameshift by an acridine half-mustard (ICR-191) (22). Moreover, mutation to operator constitutivity increased 5- to 8-fold the rate of ultraviolet-induced reversion of a frameshift or of an ochre His$^-$ mutation (23).

Possible Factors Peculiar to Starved Cells

Apart from the effect of transcription, the data summarized above on aging cultures make it clear that something occurring in stationary-phase cells, after a long delay, can greatly increase the rates of at least certain kinds of mutations. As Cairns *et al.* (1) noted, in standard bacteriological diagnostic tests "late fermenters" often appear only after an extraordinarily long delay, reflecting mutational activation of a cryptic operon rather than slow fermentation. Unfortunately, we know so little about the physiology of starved cells that we can only speculate about possible relevant factors. Among these, energy is required both for transcription and for repair of DNA—processes with opposite effects on mutation rate. Hence, the level of energy needed for an increased rate in induced operons might be achieved only late in the stationary phase, after the endogenous supply of energy has been reduced by depletion of the ribosomes and other reservoirs.

Starvation undoubtedly depletes the deoxyribonucleotide substrates and also may interfere with regulation of the ratios of the four bases. Such metabolic changes might be expected to affect both replication errors and repair of damage to DNA. Another major possibility is loss of repair enzymes, among the many changes in protein composition that have been observed.

In addition, studies of supercoiling of DNA have provided concrete evidence that variations in metabolic conditions will affect strand exposure in transcribed DNA. Transcription generates both positive and negative supercoils, and their levels are sensitive to changes in such environmental factors as aerobiosis, temperature, and osmotic regulation (24). It seems almost inescapable that some of the extensive, though ill-defined, metabolic changes in the stationary phase will also affect supercoiling and, hence, the ability of DNA to unwind.

A less likely, but possible, factor is the destruction of ribosomes. By the time cell death begins in starved *E. coli* (in liquid cultures in a minimal medium), after several days, almost no ribosomal particles can be detected; and, indeed, the complete loss of these protein-containing components (and hence loss of the ability to regenerate them) appears to be a major cause of cell death (17). Since the ribosomes translating the mRNA normally press up to the region of transcription, the depletion of ribosomes in starving cells could affect the structure of that region.

Finally, we come to the problem of the high frequency of excision of transposable elements in starving cells (7, 8). Normally the mobility of such elements is high when they infect a cell but soon subsides, because they begin to express their gene for a repressor of the transposase (25, 26). (Indeed, it is because of this self-repression that transposons have proved useful as localizable mutagens.) Depletion of the repressor could provide an obvious explanation for the restoration of rapid excision under conditions of starvation. Moreover, recent evidence suggests that precise excision involves a copy choice mechanism of recombination, which depends on single-stranded DNA at both terminal repeats (27): hence if slow transcription in starving cells created a relatively prolonged single-stranded state in one of these regions it might promote excision.

In this connection a teleonomic argument is tempting. From an evolutionary standpoint it would seem useful for DNA to become more mutable, and even more for transposable elements to become more mobile, in starving cells, thus giving rise to innovations that might promote survival of the genes of the cell or the element. Clearly, the problems raised by the papers reviewed here call for studies on the concentrations of transposase and repressor, as well as of repair enzymes, in starving cells.

Possible Limitations of the Model

Transcriptional facilitation of mutation seems an obvious explanation for the observations noted by Cairns *et al.* (1); yet, it was not suggested in any of the several published comments. The reason may be that an additional finding in that paper seemed to exclude this explanation: while the hydrolyzable inducer lactose caused Lac$^+$ mutants to accumulate, a nonhydrolyzable inducer, isopropyl β-D-thiogalactoside (IPTG), failed to do so. However, this observation may not be decisive, after all: for induction of transcription requires not only an inducer; it also requires the presence of building blocks and energy.

It seems quite possible that in starving Lac$^-$ mutants lactose, but not IPTG, might provide a low level of energy, which could be supporting sufficient transcription to result in enhanced mutability. (Lactose seems more likely than IPTG to serve as a poor substrate for enzymes other than the normal one in the cell; and the amber mutation in *lac* might be leaky.) These considerations suggest an experimental test of the mechanism proposed here: in stationary-phase cells a slow supply of energy should permit IPTG to enhance the conversion of Lac$^-$ to Lac$^+$. Indeed, a parallel experiment has already been reported: with the interrupted Ara–Lac fusions described above, incubated with arabinose and lactose, the addition of a limiting concentration of glucose increased the number of excisions (7).

Some mutations appearing in the stationary phase have one reported feature that is not obviously explained by the proposed mechanism: while the excisions that restored function are definitely located in the activated operon, in the His system many of the apparent reversions were described as suppressor mutations (28). It is not clear how increased transcription of an operon for an enzyme could stimulate mutation of a tRNA gene; but such a response would be a problem for any mechanism. Clearly, more information on the molecular nature of the substrate-induced mutations is necessary.

High-frequency genetic variation in aging cultures of *Neisseria gonorrhoeae*, leading to formation of pili with new specificity, has been traced to a very different mechanism. This organism lyses easily, releasing DNA, when growth ceases, and transformation recombines the expressed pilin gene in the recipient with silent variant pilin genes from the donor (29). However, such recombination does not seem relevant to the phenomena discussed here: not only are enterobacteria much less readily lysed, but transformation could not readily account for the precise excision of transposable elements, nor would it explain the stimulatory role of substrate.

Conclusions

Given the evidence that unknown features of stationary cultures can strikingly increase mutation rates, and especially excision of insertion sequences, induction of transcription can reasonably explain the ability of potential substrates to selectively promote adaptive mutations in starving cells. We can thus escape an artificial dichotomy: a Lamarckian inheritance of acquired characteristics (1), which violates the "central dogma" of molecular genetics, or the traditional selection by the environment among randomly mutated phenotypes, which would leave the findings unexplained.

The meaning of randomness in mutations provides the key to this escape. Used to mean an undirected process, occurring by chance in any gene, this term has become firmly embedded in classical genetics. But equally clearly, randomness in the strict mathematical sense, as equal probability, does not apply to the genome at the level of nucleotides or of short sequences surrounding a site of mutation. The notion of biased randomness is thus not a radical one: it has been with us since the discovery of fine-structure genetics, with its recognition of mutational hot spots. Here I am simply extending the notion to longer sequences, subject to influence by the environment.

But in reconciling the substrate effect with the principles of molecular genetics, this proposal does not eliminate the radical implications of that effect for evolution. For if the Darwinian process is extended from environmental selection of phenotypes to include an environmental process that in effect selects elements within the genotype for an increase in the frequency of chance mutations, the effect would simulate directed mutation: it would preferentially increase the supply of adaptive phenotypes, and these would then be subject to classical selection. But just as Monod greatly advanced the analysis of gene regulation by introducing the concept of induced enzymes, in place of the conventional concept of adaptive enzymes, clarity requires recognition that we are dealing with environmentally induced, but not necessarily directed, mutations.

It is not hard to understand, teleonomically, why adaptive mutations should be especially prominent in organisms that are not thriving. At the same time, one might expect a biasing influence of the environment to be restricted to single-celled organisms, and to a limited range of genes and functions in these; it does not support the unlimited directive process of the romantic Lamarckian vision.

The mechanism suggested by Cairns *et al.* (1) also depends, like the present proposal, on transcription, but with the mutation occurring in the mRNA rather than in the single-stranded DNA. The Lamarckian feature is the further suggestion that stabilization of the mutation in DNA somehow depends on recognition of its adaptive value. This explanation for selectivity no longer seems necessary, since his IPTG experiment can no longer be seen as decisively excluding induced transcription as an explanation. However, reversed transcription could be retained, as an alternative to direct change in the DNA, as a mechanism for biasing the random process of mutation.

The mechanism proposed here also has obvious implications for the well-known effect of cell multiplication in increasing the initiation of cancer. The conventional explanation is that replication provides increased opportunity for introducing errors in DNA. However, since growth of animal cells depends on activation of various cellular protooncogenes that produce growth factors or their receptors, transcription of these genes could also provide increased opportunity for them to undergo a carcinogenic mutation.

In this connection, Cairns (30) has summarized the strong evidence that chromosomal rearrangements play a larger role than point mutations in causing cancer; and he has further commented that the former have a more idiosyncratic molecular biology (presumably meaning subject to presently unknown and unpredictable metabolic influences). It is therefore pertinent that in stationary bacteria the presence of the potential substrate of an operon stimulated rearrangements more than it stimulated point mutations, by many orders of magnitude (8).

Because the paper of Cairns *et al.* (1) has raised such interesting conceptual challenges and such vigorous controversy it seemed worthwhile to present this theoretical discussion. Spencer Farr and Bruce Ames (personal communication) have independently developed and are testing the same hypothesis presented here.

Note Added in Proof. To test the hypothesis that a small supply of energy might allow IPTG to induce Lac^+ mutations in starved bacteria, experiments were carried out as shown in figure 3 of Cairns *et al.* (1), using the same *uvr* strain (kindly provided by J. Cairns). A stationary, overnight culture in top agar (1:5) was overlaid with 3 ml of top agar containing 0.01 M IPTG or glycerol or both, incubated for 2–3 days, and then overlaid with 0.3% lactose in top agar. IPTG alone had no effect on the appearance of Lac^+ colonies. Glycerol (0.1%) yielded an accelerated shower of colonies 1–2 days after addition of the lactose (depending on the age of the inoculum), while additional colonies subsequently appeared more slowly, as with lactose alone. Mixtures of IPTG and glycerol yielded 20–100% stimulation above that due to glycerol alone. Efforts to increase this stimulation by prolonged starvation of the cells before inoculation had the opposite effect.

The stimulatory effect of glycerol alone is evidently due to rapid cell multiplication until the glycerol is exhausted, rather than to prolonged partial starvation: the number of early colonies was not affected by a 10-fold decrease in the inoculum (as noted by Shapiro with another system; ref. 7), but it was decreased by lowering the glycerol concentration. IPTG thus evidently exerted its effects primarily on a brief wave of growing cells, rather than on starving cells. Accordingly, the procedure used does not appear to have provided a sharp test for the postulated effect of slow transcription, in semistarving cells, on mutation rate.

Since commercial lactose ordinarily contains a trace of glucose the possibility had to be considered that this contaminant might be providing energy required for the slow emergence of Lac^+ colonies in the presence of lactose. However, the substantial amount of glycerol required for an effect, and its transient action, suggested that if an exogenous energy supply is required for the response to lactose it must be derived from the lactose itself. Moreover, further experiments showed that preincubating the lactose with glucose oxidase, or with a suspension of cells of the Lac^- mutant, did not alter the numbers of colonies emerging in its presence.

How lactose promotes the emergence of Lac^+ mutants thus remains open: whether by supporting multiplication over several days similar to that produced by glycerol over several hours; by supporting very slow growth accompanied by an elevated mutation rate of the induced operon, as suggested in this paper; or by yet another mechanism. But it does seem clear that these reversions or suppressions of an amber mutation in *lac* are much more frequent in growing cells, and hence much less dependent on starvation, than the reported excision of insertion sequences.

I am indebted to John Cairns for helpful comments. B.D.D. is a Scholar-in-Residence, Fogarty International Center, National Institutes of Health, where research facilities were generously provided by Dr. Herbert Tabor.

1. Cairns, J., Overbaugh, J. & Miller, S. (1988) *Nature (London)* **335,** 142–145.
2. Stahl, F. W. (1988) *Nature (London)* **335,** 112–113.
3. Cairns, J. (1988) *Nature (London)* **336,** 527–528.
4. Ryan, F. J. (1955) *Genetics* **40,** 726–738.
5. Hall, B. G. & Clarke, N. D. (1977) *Genetics* **85,** 193–201.
6. Hall, B. G. (1982) *J. Bacteriol.* **151,** 269–273.
7. Shapiro, J. (1984) *Mol. Gen. Genet.* **194,** 79–90.
8. Hall, B. G. (1988) *Genetics* **120,** 887–897.
9. Benson, S. A. (1988) *Nature (London)* **336,** 21–22.
10. Ryan, F. J., Nakada, D. & Schneider, M. (1961) *Z. Vererbungsl.* **92,** 38–41.
11. Ryan, F. J., Okada, T. & Nagata, T. (1963) *J. Gen. Microbiol.* **30,** 193–199.
12. Postgate, J. R. & Hunter, J. R. (1962) *J. Gen. Microbiol.* **29,** 233–262.
13. Ingraham, J. L., Maaloe, O. & Neidhardt, F. C. (1983) *Growth of the Bacterial Cell* (Sinauer, Sunderland, MA).
14. Groat, R. G., Schultz, J. E., Zychlinsky, E., Bockman, A. & Matin, A. (1986) *J. Bacteriol.* **168,** 486–493.
15. Martin, J. F. & Demain, A. L. (1980) *Microbiol. Rev.* **44,** 230–251.
16. Connell, N., Han, Z., Moreno, F. & Kolter, R. (1987) *Mol. Microbiol.* **1,** 195–201.

17. Davis, B. D., Luger, S. M. & Tai, P. C. (1986) *J. Bacteriol.* **166,** 439–445.
18. Siebenlist, U., Simpson, R. B. & Gilbert, W. (1980) *Cell* **20,** 269–281.
19. Lindahl, T. & Neiberg, B. (1972) *Biochemistry* **11,** 3610–3618.
20. Lindahl, T. & Neiberg, B. (1974) *Biochemistry* **13,** 3405–3410.
21. Singer, B. & Kusmierek, J. T. (1982) *Annu. Rev. Biochem.* **52,** 655–693.
22. Herman, R. K. & Dworkin, L. B. (1971) *J. Bacteriol.* **106,** 543–550.
23. Savic, D. J. & Kanazir, D. T. (1972) *Mol. Gen. Genet.* **118,** 45–50.
24. Liu, L. F. & Wang, J. C. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7024–7027.
25. Chou, J., Lemaux, P. G., Casadaban, M. J. & Cohen, S. N. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4020–4024.
26. Heffron, F., McCarthy, B. J., Ohtsubo, H. & Ohtsubo, E. (1979) *Cell* **18,** 1153–1164.
27. Brunier, D., Michel, B. & Ehrlich, S. D. (1988) *Cell* **52,** 883–892.
28. Savic, D. J. & Kanazir, D. T. (1975) *Mol. Gen. Genet.* **137,** 143–150.
29. Seifert, H. S., Ajioka, R. S., Marchal, C., Sparling, P. F. & So, M. (1988) *Nature (London)* **336,** 393–395.
30. Cairns, J. (1981) *Nature (London)* **289,** 353–357.